

STUDY TITLE

Optimum® AcreMax™ 1 and Optimum® AcreMax™ RW Field Performance and Rootworm Damage
Thresholds

DATA REQUIREMENTS

None

AUTHORS

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STUDY COMPLETION DATE

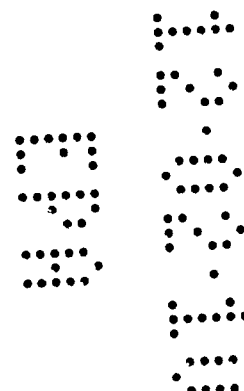
November 29, 2010

PERFORMING LABORATORIES

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STUDY NUMBER

PHI-2010-240



STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

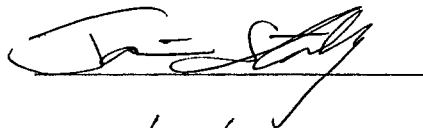
No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C). *

Company: Pioneer Hi-Bred International, Inc.

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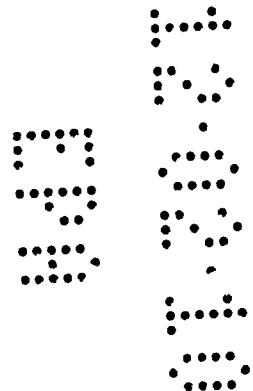
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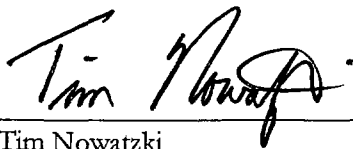


*In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.



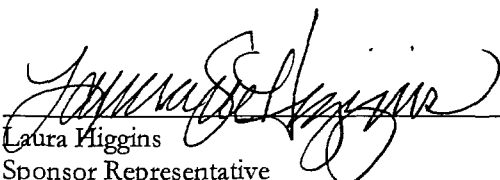
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was not conducted in accordance with the requirements for the U.S. EPA Good Laboratory Practice (GLP) Standards, 40 CFR part 160, 1989.



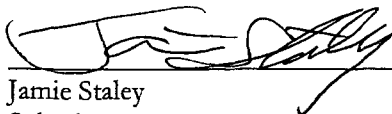
Tim Nowatzki
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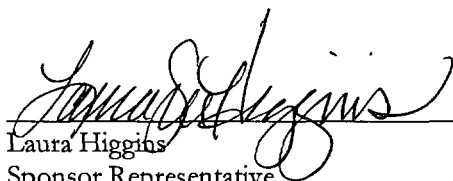
CERTIFICATION PAGE

We, the undersigned, declare that this report accurately represents the results observed during the course of this study.



Tim Nowatzki
Study Plan Coordinator
Pioneer Hi-Bred International, Inc.

11/29/10
Date



Laura Higgins
Sponsor Representative
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ABSTRACT

Pioneer has conducted several analyses of its Optimum® AcreMax™¹ Insect Protection (OAM1) and Optimum® AcreMax™ RW Insect Protection (OAMRW) field performance data and has developed OAM1 and OAMRW-specific field performance criteria so that unexpected damage can be benchmarked. These performance criteria and associated root damage thresholds, along with the implementation of the Sublethal Seedling Assay for monitoring changes in rootworm susceptibility to the Cry34/35Ab1 proteins, and the current resistance monitoring and remedial action plans specified in the Terms and Conditions of the OAM1 and OAMRW registrations (EPA Registration Nos. 29964-6 and 29964-10), constitute the OAM1 and OAMRW-specific resistance monitoring and remedial action plans.

INTRODUCTION

On September 29, 2010, the United States Environmental Protection Agency granted registration extensions to Pioneer Hi-Bred International, Inc. (Pioneer) for Optimum® AcreMax™ 1 Insect Protection (OAM1) and Optimum® AcreMax™ RW Insect Protection (OAMRW) integrated rootworm refuge *Bt* corn products. A condition of the registrations was for Pioneer to provide detailed OAM1 and OAMRW-specific resistance monitoring and remedial action plans, including an analysis to determine the expected field performance criteria for the OAM1 and OAMRW products so that unexpected damage could be benchmarked. Pioneer has conducted several analyses of its OAM1 and OAMRW field performance data collected from field plots across multiple years which contained mixed populations of western (*Diabrotica virgifera virgifera*) and northern (*Diabrotica barberi*) corn rootworm to develop OAM1 and OAMRW-specific field performance criteria. These performance criteria, along with the implementation of the Sublethal Seedling Assay for monitoring changes in corn rootworm susceptibility to event DAS-59122-7, and the current resistance monitoring and remedial action plans specified in the Terms and Conditions of the OAM1 and OAMRW registrations (EPA Registration Nos. 29964-6 and 29964-10), constitute the OAM1 and OAMRW-specific resistance monitoring and remedial action plans.

EXPECTED PERFORMANCE OF ACREMAX 1 AND ACREMAX RW UNDER COMMERCIAL USE

DAS-59122-7 Performance

In March 2010, Pioneer submitted a comprehensive analysis of the field performance of the rootworm trait in the OAM1 and OAMRW products (event DAS-59122-7) when planted without integrated refuge (MIRID No. 480455-01) which confirmed that the trait provides reliable plant protection. However, it was also noted that many environmental and operational factors contribute to the actual amount of root feeding and stalk-/root-lodging observed in DAS-59122-7 plants in the field. These factors included:

Insect factors:

- Rootworm population and egg distribution
- Species composition
- Ability of rootworm to identify suitable host tissue
- Chronic effects of the Cry34/35Ab1 proteins on rootworm larvae
- Density dependent competition for feeding sites

Plant factors:

- Size of root mass at time of rootworm feeding
- Re-growth of corn roots following feeding
- Phenotypic variation in expression of Cry34/35Ab1 proteins
- Temporal and spatial pattern of Cry34/35Ab1 protein concentration in corn roots

Field factors:

- Field history (previous crop)
- Soil conditions (e.g. moisture at egg hatch), structure, type, fertility
- Root damage from agents other than corn rootworm
- Presence of pathogens and predators

Weather factors:

- Winter conditions
- Rain and weather conditions during rootworm egg hatch and larval feeding period
- Temperature effects on egg hatch
- Environmental impacts on corn growth and development

Management factors:

- Use of insecticides, fertilizers
- Cultivation, planting conditions
- Presence of weeds, volunteers, alternate hosts

As described in the report, field performance of DAS-59122-7 in pure stands (non-integrated refuge) can be variable and impacted by a wide range of factors in the absence of resistance.

OAM1 and OAMRW Performance Mirrors that of DAS-59122-7 in Pure Stands

Pioneer has conducted multi-year studies to investigate the field performance of integrated refuge products including OAM1 and OAMRW. Of particular interest in this line of investigation was characterizing the performance of *Bt* rootworm protected plants located in close proximity to integrated refuge plants. In developing the expected field performance criteria for OAM1 and OAMRW products, Pioneer investigated whether *Bt* rootworm-protected plants in close proximity to refuge plants incurred more root damage than *Bt* rootworm-protected plants in a pure stand. Results from this analysis are beneficial in determining how to evaluate root damage to DAS-59122-7 plants in a field with integrated refuge.

Analysis of Root Damage to Bt Rootworm Protected Plants in Close Proximity to Integrated Refuge Plants

Summary of OAM1 Corn Rootworm Efficacy Experiments

a. 2008 Research Experiment and Small Demonstration Plots

The Pioneer Research experiment was conducted at three locations (Johnston, IA; Mankato, MN; Rochelle, IL) in 2008. Plots were located on land that was corn the previous season with a history of natural infestations of western and northern corn rootworms (Mankato and Rochelle locations were corn rootworm trap crops the previous season). Additionally, plants in each treatment were manually infested with 1,000 western corn rootworm eggs per plant at plant growth stage V2-V3. Treatments included integrated refuge plots at a 10% blend rate (3 refuge plants out of 30 plants per row), pure stand DAS-59122-7 plots, and pure stand refuge plots, each in three hybrid backgrounds. Treatments were arranged in a randomized complete block design with three blocks at each location. Each plot was a single-row 17.5' in length, and three clusters of 5 plants each were infested, dug and scored using the Iowa State 0-3 node-injury scale (CRWNIS, Oleson et al. 2005). For integrated refuge plots, each cluster contained one refuge plant and two rootworm protected plants on either side of the refuge plant. A small demonstration plot was imbedded within the experiment at each location. The plot was seven-rows wide, 17.5' long and each row contained one refuge plant out of 30 (a 3.3% integrated refuge rate). All 210 plants from this demonstration plot were infested, dug and scored for root injury. All seeds in all treatments were treated with clothianidin, an insecticidal seed treatment, at a rate of 0.25 a.i. per kernel. This is the labeled rate for control of secondary insect pests of maize and is not labeled for corn rootworm control.

This data set was particularly useful for observing patterns of rootworm damage on DAS-59122-7 protected plants closer or further away from an integrated refuge plant. The level of corn rootworm pressure when pooled across these locations would be classified as moderate, with CRWNIS scores of pure stand refuge plants (PS-R) averaging just over 1 node injured across the 3 locations (Figure 1). CRWNIS score distributions for different types of plants (based on distance from a blended refuge plant) and descriptive statistics (mean, median, standard deviation, and 95th percentile) are provided in Figure 1. The distributions of CRWNIS scores for pure stand refuge and integrated refuge plants were relatively similar (Figure 1). Additionally, the distributions of CRWNIS scores for all *Bt*-protected plants were relatively similar regardless of distances from a blended refuge plant, and the CRWNIS scores for DAS-59122-7 plants were significantly lower than those from refuge plants.

b. 2008 Agronomy Science Experiment

The Agronomy Science experiment was conducted at ten locations (Breda, IA; Orange City, IA; Johnston, IA; Readlyn, IA; Janesville, WI; Mankato, MN; Princeton, IL; Rochelle, IL; Windfall, IN; Windfall, IN(2)) in 2008. Plots were located on land that was corn the previous season with a history of natural infestations of western and northern corn rootworms. Treatments included integrated refuge plots at 3%, 6%, and 9% blend rates, pure stand DAS-59122-7 plots, and pure stand refuge plots. Treatments were arranged in a randomized complete block design with four blocks at each location. Each plot was a four-row plot, 17.5' in length, and a cluster of 5 plants from row 1 or row 4 of each plot was dug and scored using the Iowa State 0-3 node-injury scale. For integrated refuge plots, a cluster contained one refuge plant and two rootworm *Bt*-protected plants on either side of the refuge plant. All seeds in all treatments were treated with insecticidal seed treatments. The pure stands of DAS-59122-7 were treated with clothianidin at a rate of 0.25 mg a.i. per kernel, whereas the refuge pure stands were treated with either thiamethoxam at a rate of 0.50 mg a.i. per kernel or clothianidin at a rate of 1.25 a.i. per kernel. Two seed treatment combinations were tested in the integrated refuge treatments. The integrated refuge seeds were treated with either thiamethoxam at a rate of 0.50 mg a.i. per kernel or clothianidin at a rate of 1.25 a.i. per kernel; while the corn rootworm protected portion of the integrated refuge treatments was treated with clothianidin at a rate of 0.25 a.i. per kernel. The thiamethoxam and low rate clothianidin seed treatments are labeled for control of certain secondary pests of maize while the high-rate clothianidin seed treatment is labeled for control of corn rootworm.

This data set was also useful for observing patterns of rootworm damage on DAS-59122-7 plants neighboring (1 away, 2 away) a refuge plant versus pure stand DAS-59122-7 plants further away from refuge plants. Data distributions for different plant types and descriptive statistics are provided in Figure 2. Although there was a numerical trend of decreasing root damage (CRWNIS scores) on plants further away from an integrated refuge plant, no statistically significant differences were observed between different types of *Bt* protected plants (neighboring versus pure stand) at most sites with adequate root worm pressure.

c. 2009 Mini-strip Experiment

The mini-strip experiment was conducted at five locations (Johnston, IA; Mankato, MN; Champaign, IL; Windfall, IN; York, NE) in 2009. Plots were located on land that was corn the previous season and had a history of containing natural infestations of western and northern corn rootworms. Additionally, plants in each treatment were manually infested with western corn rootworm eggs. Treatments included integrated refuge plots at 5% and 10% blend rates, pure stand DAS-59122-7 plots, pure stand refuge plots, and pure stand refuge plots treated with a granular insecticide applied at planting time (Force®3G, 4 oz. per 1,000 ft. of row). Treatments were arranged in a randomized

complete block design with three blocks at each location. Each plot was eight-rows wide, 52.5' in length, and each row contained approximately 100 plants. Four clusters of 5 plants each from rows 2 and 7 (eight clusters in total) of each plot were infested, dug and scored using the Iowa State 0-3 node-injury scale. Clusters in row 2 were infested with 500 western corn rootworm eggs per plant and clusters in row 7 were infested with 1,000 western corn rootworm eggs per plant. For integrated refuge plots, each cluster contained one refuge plant and two rootworm protected plants on either side of the refuge plant. The insecticidal seed treatment thiamethoxam, at a rate of 0.25 mg a.i. per kernel was applied to all seeds in all entries and is the labeled rate for control of certain secondary pests of maize.

This data set was useful for observing patterns of rootworm damage in larger field plots. Data distributions for different plant types and descriptive statistics are provided in Figure 3. The distribution of CRWNIS scores for *Bt* rootworm-protected plants neighboring refuge plants were similar to those in the pure stand *Bt* rootworm-protected plots regardless of their proximity to integrated refuge plants.

Damage Thresholds for OAM1 and OAMRW Products Should be the Same as for DAS-59122-7 Pure Stand Products

EPA's review of report MIRID No. 480455-01 as summarized in the Cry34/35Ab1 Corn Biopesticides Registration Action Document contains EPA's recommendation that a "damage threshold level" be implemented for rootworm traits and emphasized it is one tool that should not be used alone or out of context with the many factors that can affect rootworm trait performance. EPA suggested a damage threshold level of 1.0 on the Iowa State node-injury scale may be suitable for a single toxin, non-high dose CRW rootworm product such as DAS-59122-7 (under exceedingly high rootworm pressure, BPPD suggests a damage threshold level of 1.5 may be appropriate). Pioneer agrees that these levels would indicate unexpected damage regardless of the DAS-59122-7 expressing product. Pioneer's analysis of the currently available root damage ratings for OAM1 and OAMRW products supports that DAS-59122-7 plants in the OAM1 and OAMRW products will perform like DAS-59122-7 plants in fields without integrated refuge. Therefore, an OAM1 or OAMRW field identified as having an overall average CRWNIS rating of 1.0 or greater for plants containing event DAS-59122-7 (1.5 or greater under exceedingly high rootworm pressure) where all other environmental and operational factors have been ruled out as influencing product performance will be identified as a case of unexpected damage and will be investigated as outlined in the "Suspected Resistance – Investigation of Field Reports" section of the Terms and Conditions of the OAM1 and OAMRW registrations.

RESPONSE PLAN FOR INVESTIGATING PERFORMANCE INQUIRIES

All customer inquiries on the performance of the rootworm trait in OAM1 and OAMRW products will be handled through the following internal processes:

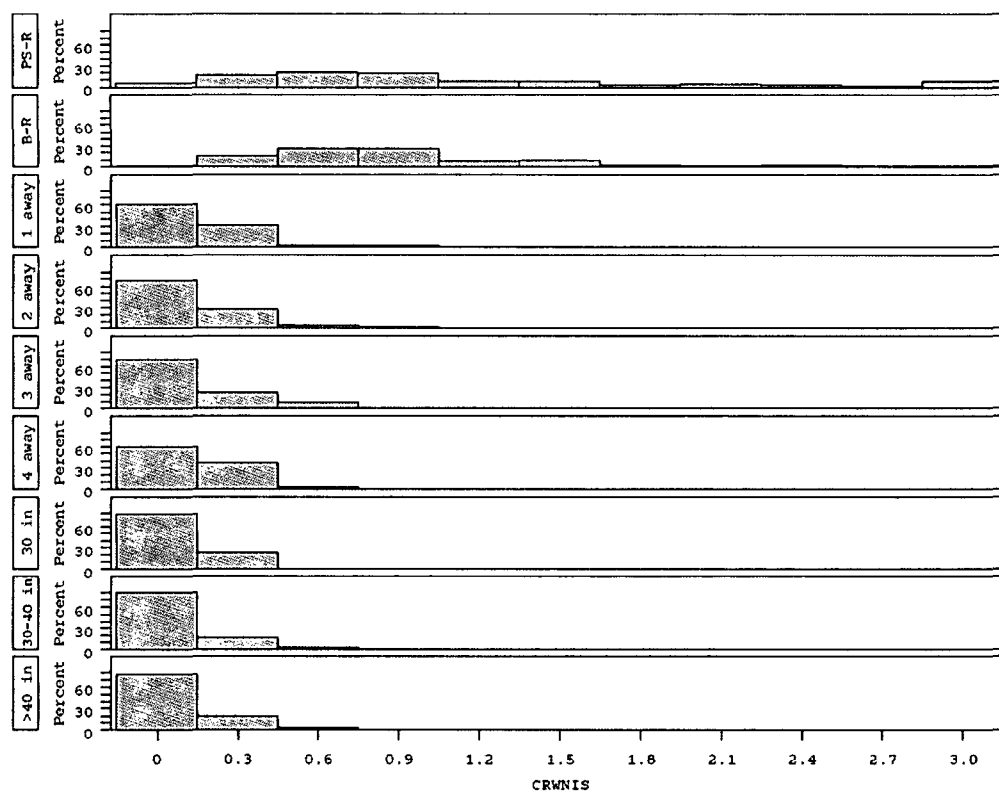
1. Interviews are conducted and a field visit is arranged as soon as reasonably possible following a customer complaint. The visit involves walking through the entire area in question to examine damage (e.g. lodging, stunting, or other symptoms) to the OAM1 or OAMRW product.
2. Damage found is assessed for causative agent, be it corn rootworm, other insects, adverse weather, planting errors, or other factors.

3. If causative agents other than corn rootworm do not clearly explain the damage, the field is investigated for rootworm damage by sampling and evaluating root masses using a sequential sampling plan developed for OAM1 and OAMRW products. The sampling plan includes evaluating root masses from plants that contain event DAS-59122-7 (identified by ELISA trait check kits specific to the Cry34/35Ab1 proteins). Tissue samples may be collected for quantitative ELISA in the laboratory.
4. If the field in question has an overall average CRWNIS rating of 1.0 or greater for plants containing event DAS-59122-7 (1.5 or greater under exceedingly high rootworm pressure) where all other environmental and operational factors have been ruled out as influencing product performance, the field will be identified as a case of unexpected damage and will be investigated as outlined in the "Suspected Resistance – Investigation of Field Reports" section of the Terms and Conditions of the OAM1 and OAMRW registrations. This investigation will include the collection of rootworm adults (if present), quantifying Cry34/35Ab1 levels in the damaged plants, and subsequent steps for suspected resistance. In the event corn rootworm beetles are not present in the field at the time of evaluation, an attempt to collect beetles from the same area the following year will be made.
5. Collection of adults in sufficient numbers is intended for harvesting eggs in the laboratory which, after an obligate diapauses period, will be used in the Sublethal Seedling Assay to determine population susceptibility to DAS-59122-7.

CONCLUSIONS

Pioneer has conducted several analyses of its OAM1 and OAMRW field performance data collected to date and has developed OAM1 and OAMRW-specific field performance criteria so that unexpected damage to DAS-59122-7 plants deployed with integrated refuge can be benchmarked. Efficacy data collected to date indicate that average CRWNIS scores to DAS-59122-7 plants deployed with integrated refuge will be similar to DAS-59122-7 plants deployed in a pure stand planting. These performance criteria and associated root damage thresholds, along with the implementation of the Sublethal Seedling Assay for monitoring changes in rootworm susceptibility to the Cry34/35Ab1 proteins, and the current resistance monitoring and remedial action plans specified in the Terms and Conditions of the OAM1 and OAMRW registrations (EPA Registration Nos. 29964-6 and 29964-10), constitute the OAM1 and OAMRW-specific resistance monitoring and remedial action plans.

FIGURES

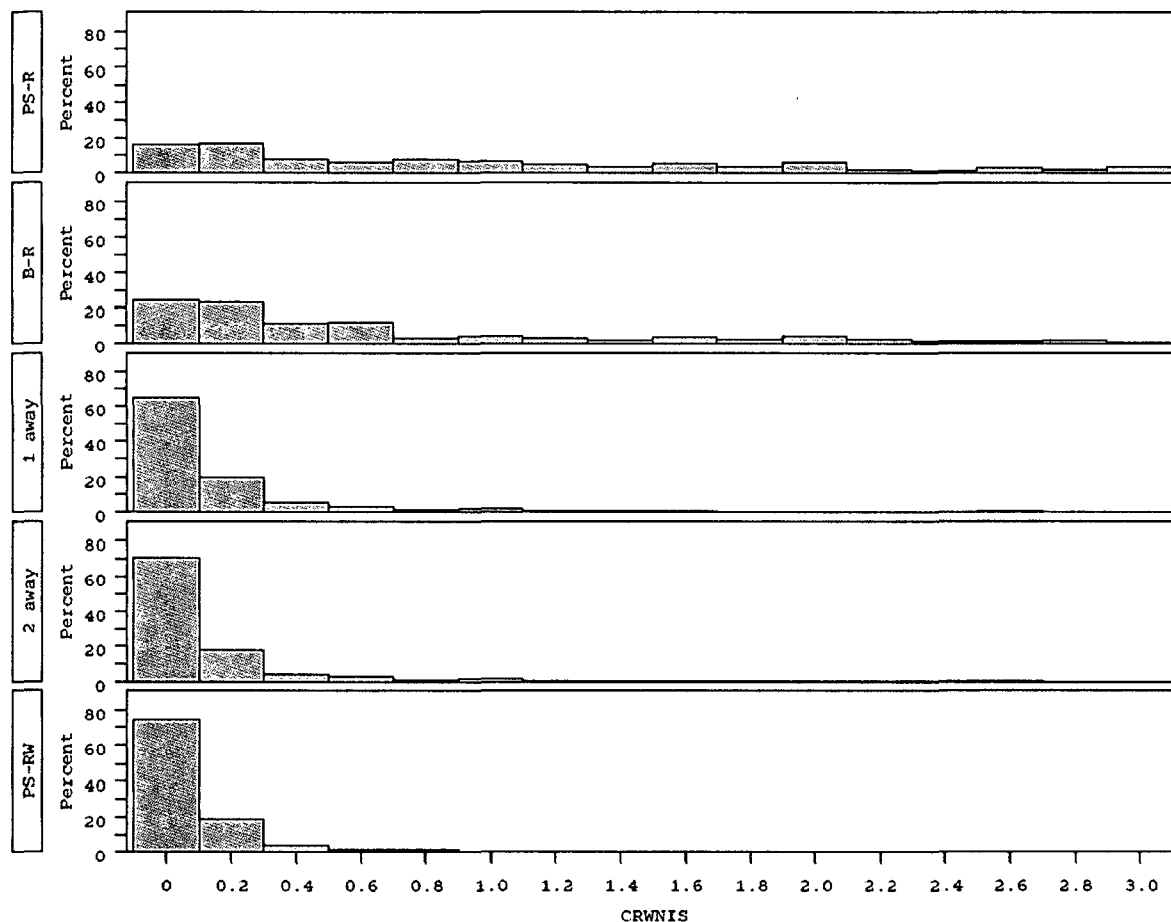


The MEANS Procedure

Analysis Variable: CRWNIS/CRWNIS					
plant type4	N	Mean	Median	Std Dev	95th Pctl
PS-R	401	1.10	0.82	0.84	3.00
B-R	85	0.99	0.78	0.66	2.47
1 away	170	0.18	0.09	0.22	0.56
2 away	170	0.15	0.08	0.17	0.46
3 away	40	0.15	0.09	0.14	0.48
4 away	40	0.16	0.08	0.13	0.41
30 in	26	0.12	0.08	0.10	0.31
30-40 in	380	0.11	0.08	0.12	0.36
>40 in	818	0.11	0.08	0.12	0.36

*Proximities of the *Bt* plants included those adjacent to (1 away), two plants away (2 away), three plants away (3 away), and four plants away (4 away) from blended refuge plants. In addition, frequency distributions of *Bt* plants located 30-inches away (either within or across rows) from blended refuge plants (30 in), between 30 and 40 inches away (30-40 in) and greater than 40 inches away from blended refuge plants (>40 in) were also computed. *Bt* plants located greater than 40 inches away from blended refuge plants represent the distribution of DAS-59122-7 root scores in a pure stand planting.

Figure 1. Frequency distributions of CRWNIS scores and summary statistics for the Pure Stand Refuge plants (PS-R), Blended Refuge plants (B-R), and DAS-59122-7 plants (*Bt* plants) at various proximities to the blended refuge plants in the 2008 Research Experiment and Small Demonstration Plots.

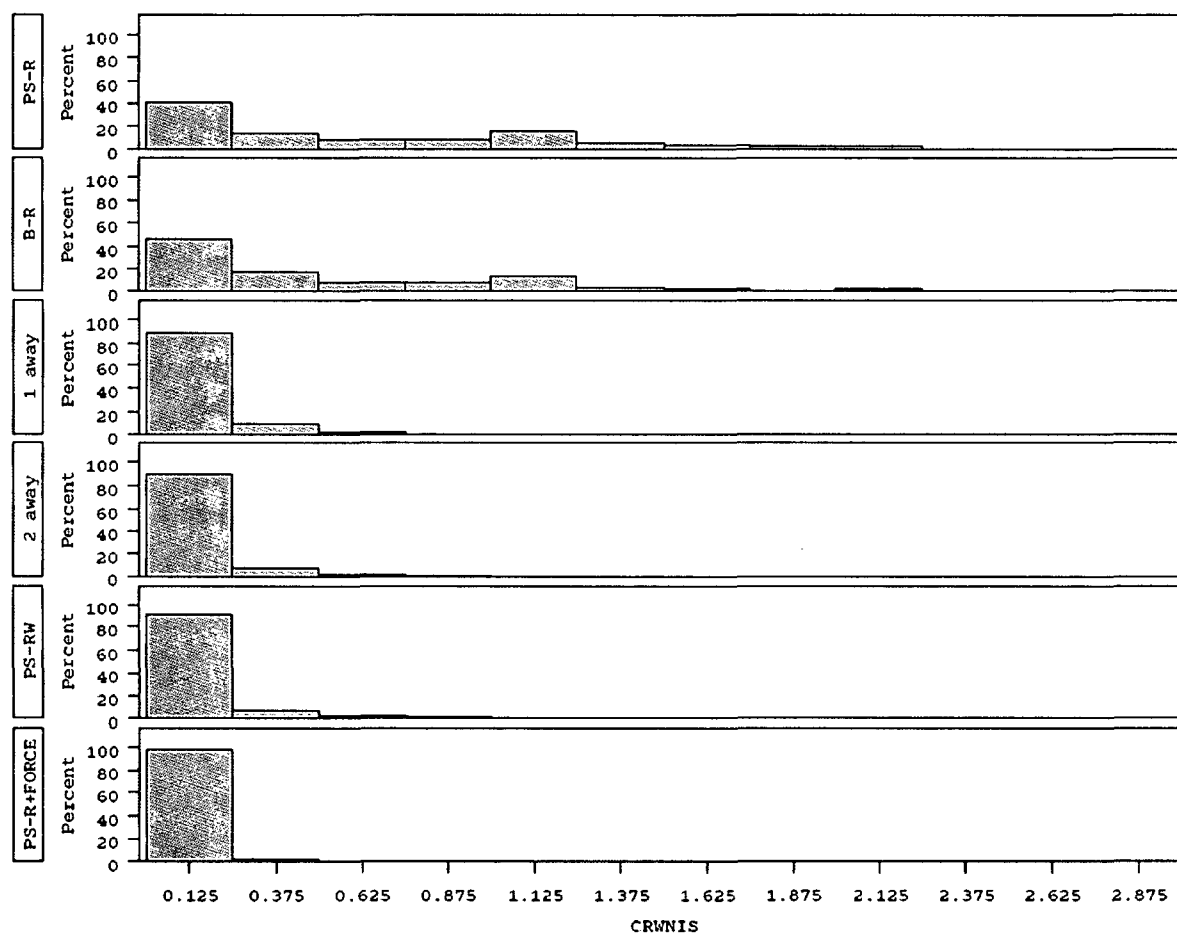


The MEANS Procedure

Analysis Variable: CRWNIS CRWNIS					
plant type4	N	Mean	Median	Std Dev	95th Pctl
PS-R	794	0.97	0.75	0.89	2.75
B-R	477	0.62	0.30	0.71	2.20
1 away	952	0.20	0.09	0.36	0.90
2 away	953	0.17	0.09	0.33	0.70
PS-RW	400	0.12	0.09	0.18	0.30

*Proximities of the *Bt* plants included those adjacent to (1 away), and two plants away (2 away) from blended refuge plants. In addition, frequency distributions of *Bt* plants in a pure stand (PS-RW) were also computed.

Figure 2. Frequency distributions of CRWNIS Scores and summary statistics for the Pure Stand Refuge plants (PS-R), Blended Refuge plants (B-R), and DAS-59122-7 plants (*Bt* plants) at various proximities to the blended refuge plants in the 2008 Agronomy Science Experiment.



The MEANS Procedure

Analysis Variable: CRWNIS CRWNIS					
plantatype4	N	Mean	Median	Std Dev	95th Pctl
PS-R	591	0.59	0.40	0.55	1.67
B-R	112	0.49	0.32	0.49	1.38
1 away	222	0.10	0.05	0.13	0.36
2 away	217	0.10	0.05	0.14	0.40
PS-RW	1183	0.10	0.05	0.13	0.36
PS-R+FORCE	587	0.07	0.07	0.05	0.17

*Proximities of the *Bt* plants included those adjacent to (1 away), and two plants away (2 away) from blended refuge plants. In addition, frequency distributions of *Bt* plants in a pure stand (PS-RW) and Pure Stand Refuge plants treated with a granular soil insecticide (PS-R+Force) were also computed.

Figure 3. Frequency distributions of CRWNIS Scores and summary statistics for the Pure Stand Refuge plants (PS-R), Blended Refuge plants (B-R), and DAS-59122-7 plants (*Bt* plants) at various proximities to the blended refuge plants in the 2009 Mini-Strip Experiment.

REFERENCE

Oleson, J.D., Y.L. Park, T.M. Nowatzki, and J.J. Tollefson. 2005. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98: 1-8.

ARCHIVING STATEMENT

Original or exact copies of all raw data and pertinent information, including the original study plan, any amendments, and the final report will be archived at:

Pioneer Hi-Bred International, Inc.
Regulatory Group Archives
2450 SE Oak Tree Court
Ankeny, IA 50021